

***Methanococcus maripaludis* medium – McCas, Nitrogen-free (N-free) or Formate**

Note: For Formate media, use underlined quantities.

Have tubes and /or bottles and stoppers in anaerobic chamber at least 1 hour ahead of time. For plates, have petri dishes in chamber at least 1 day before.

	200 ml (Tubes - Make in 0.5L flask)	500 ml (Plates - Make in 1 L flask)
Combine before boiling/ autoclaving		
H ₂ O	100 ml (<u>80</u> ml)	250 ml (<u>200</u> ml)
N-Free General Salts Soln	100 ml	250 ml
NaHCO ₃		
For liquid (hi pressure)	1 g	NA
For agar (low pressure)	NA	1 g
NH ₄ Cl (for non N-free)	0.1 g	0.25 g
NaCl	4.4 g (<u>2.1</u> g)	11 g (<u>5.25</u> g)
K ₂ HPO ₄ Soln	2 ml	5 ml
FeSO ₄ Soln	1 ml	2.5 ml
N-free Trace Minerals (1,000X)	0.2 ml	0.5 ml
Vitamin Soln (100X)	2 ml	5 ml
Rasazurin Soln	0.2 ml	0.5 ml
Na Acetate•3H ₂ O	0.28 g	0.7 g
Difco Casamino Acids (for non N-free)	0.4 g	1 g
<u>*2M MOPS</u> (pH 7)	<u>20</u> ml	<u>50</u> ml
<u>Na formate</u>	<u>2.72</u> g	<u>6.8</u> g
Difco Noble Agar	NA	7.5 g
Add just before boiling or autoclaving		
Dithiothreitol	0.1 g	0.25 g

*To Make 200 ml of 2M MOPS buffer (pH 7) – Add 83.7 g MOPS acid to 80 ml H₂O. Add approx. 25 NaOH pellets and then check pH. Then follow with incremental addition of individual pellets until buffer is neutral. Fill to 200 ml.

For making tubes:

Heat under a stream of N_2CO_2 until the rasazurin turns colourless. Continued heating will precipitate salts but it will re-dissolve upon cooling. Allow cooling under N_2CO_2 and ensure that all salts are re-dissolved. Then stopper the flask and bring into AnO_2 hood for distribution into Balch tubes (5 ml per tube). Stopper and bring out of hood for crimping. Then gas exchange headspace in tubes three times with H_2CO_2 (McCas) or N_2CO_2 (N-free or Formate). Keep final pressure at 30 psi for storage. Before inoculation, depressurize with needle to 1 atmosphere, reduce with 0.1 ml of 2.5% w/v $\text{NaS}\cdot 9\text{H}_2\text{O}$ and then add culture. Then pressurize to 40 psi H_2CO_2 (McCas and N-free).

For making Agar:

After autoclaving for 1 hour, allow to cool under a stream of N_2CO_2 gas in a 55 °C waterbath. Add antibiotics if needed. Then stopper flask and bring into the AnO_2 hood. Pour the agar onto plates and allow to cool and solidify. There will be some condensation. Put plates into incubation vessel with desiccant and also a beaker containing paper towel soaked with 10 ml of 25% w/v $\text{NaS}\cdot 9\text{H}_2\text{O}$. Close vessel. Allow to incubate over night at RT before using plates.

Formula Weights:

MOPS acid: 209.26

Na Formate: 68.02